Supplemental items

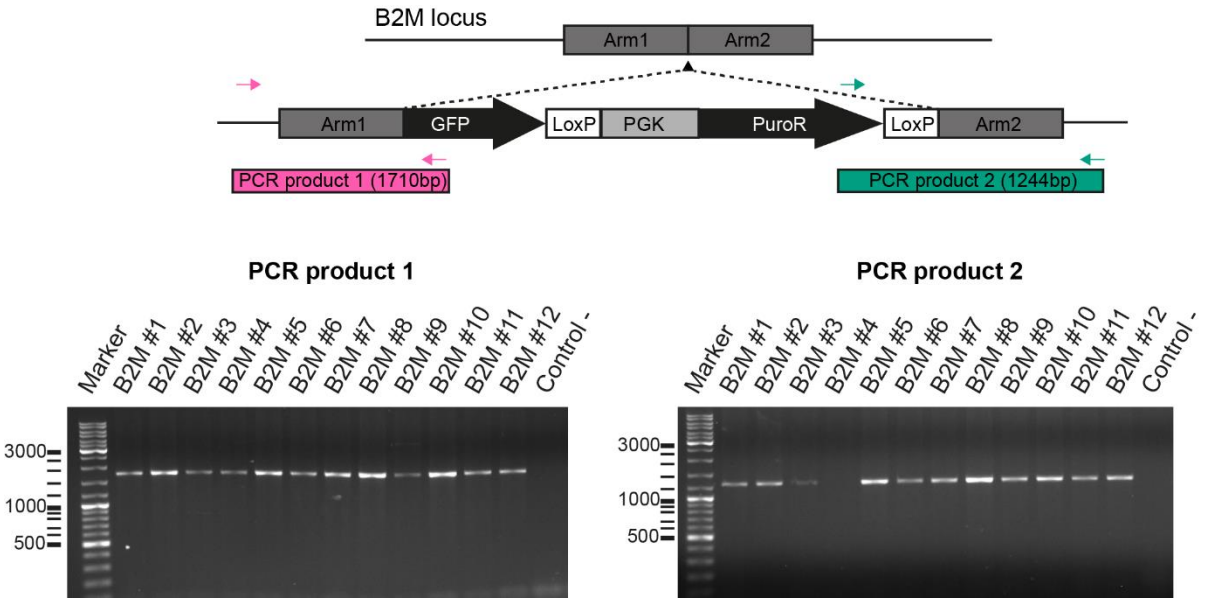

Figure S1. Genomic validation of B2M targeted clones

Overview of the genomic PCR performed on all B2M targeted clones with the primer pairs indicated on top. Unmodified control iPSCs were used as negative control (Control -).

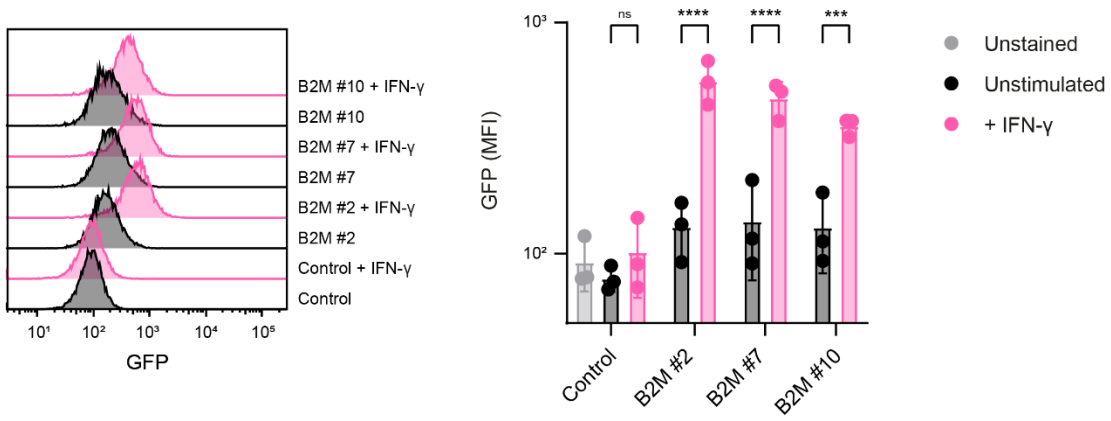

Figure S2. GFP expression in B2M targeted clones

GFP protein expression was measured by flow cytometry. Unmodified control iPSCs were used as negative control and for all cell lines results are shown for unstimulated and IFN-γ stimulated cells (n = 3 independent experiments).

Results are shown as mean ± SD and significance was evaluated using one-way ANOVA with Šidák's correction for multiple testing, comparing each sample to its own unstimulated control (ns = not significant, *** p<0.001, **** p<0.0001).

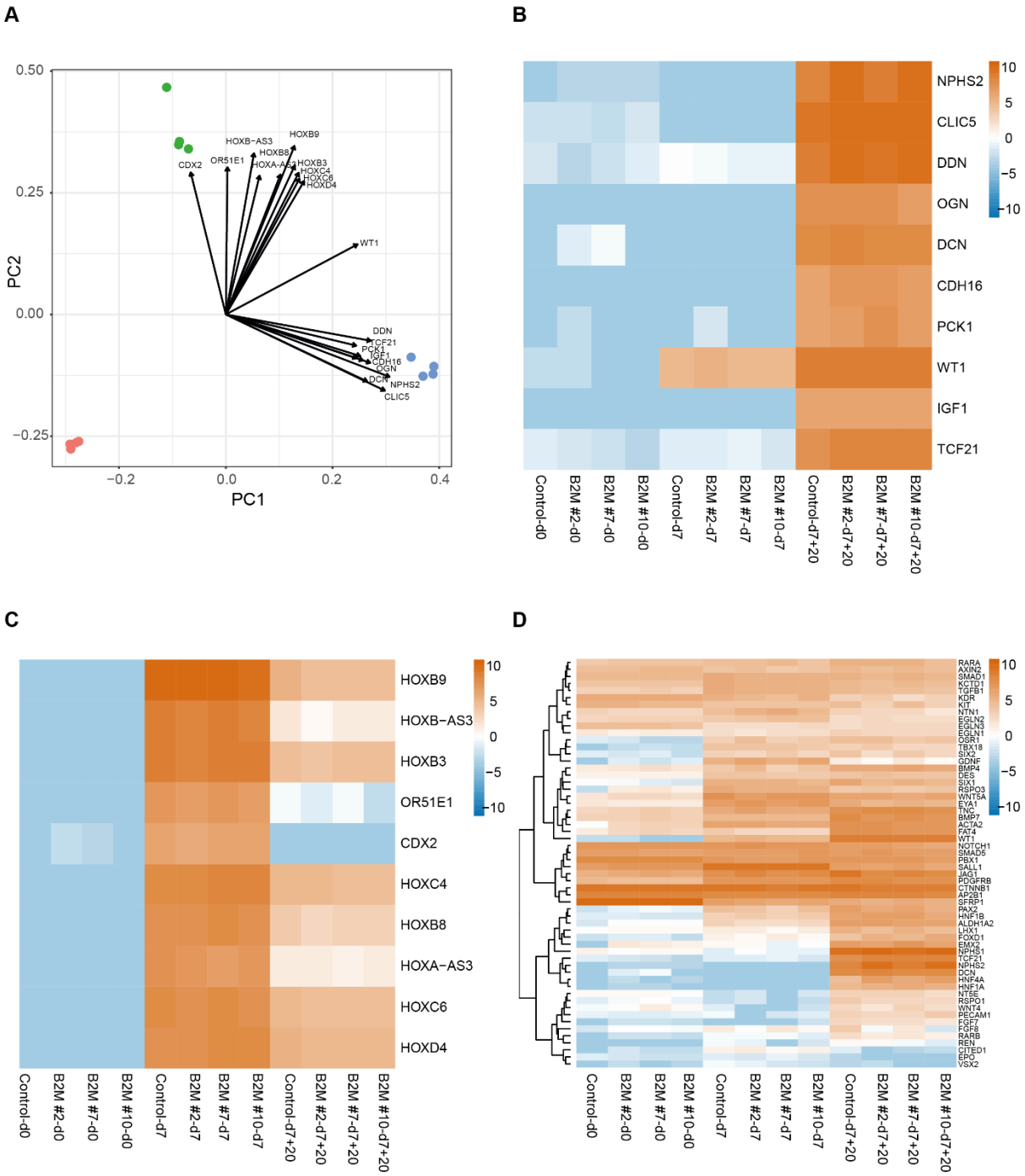

Figure S3. Detailed analysis of bulk RNA sequencing to study expression of genes during kidney organoid differentiation

A) Principal component analysis plot (similar as Figure 2B) with loadings of the top ten genes contributing to principal component 1 (*NPHS2, CLIC5, DDN, OGN, DCN, CDH16, PCK1, WT1, IGF1 and TCF21*) and 2 (*HOXB9, HOXB-AS3, HOXB3, OR51E1, CDX2, HOXC4, HOXB8, HOXA-AS3, HOXC6* and *HOXD4*). Red dots display day 0 samples, green dots day 7 samples and blue dots day 7 + 20 samples.

B) Expression levels (counts per million, cpm) for the top ten genes based on factor loadings contributing to PC1 (*NPHS2, CLIC5, DDN, OGN, DCN, CDH16, PCK1, WT1, IGF1* and *TCF21*). Heatmap was generated based on cpm based on factor loadings contributing to principal component 1.

C) Expression levels (cpm) for the top ten genes based on factor loadings contributing to PC2 (*HOXB9, HOXB-AS3, HOXB3, OR51E1, CDX2, HOXC4, HOXB8, HOXA-AS3, HOXC6* and *HOXD4*). Heatmap was generated based on cpm based on factor loadings contributing to principal component 2.

D) Expression heatmap for markers of kidney cell lineage. Genes were selected from PathCards, pathway unification database [1].

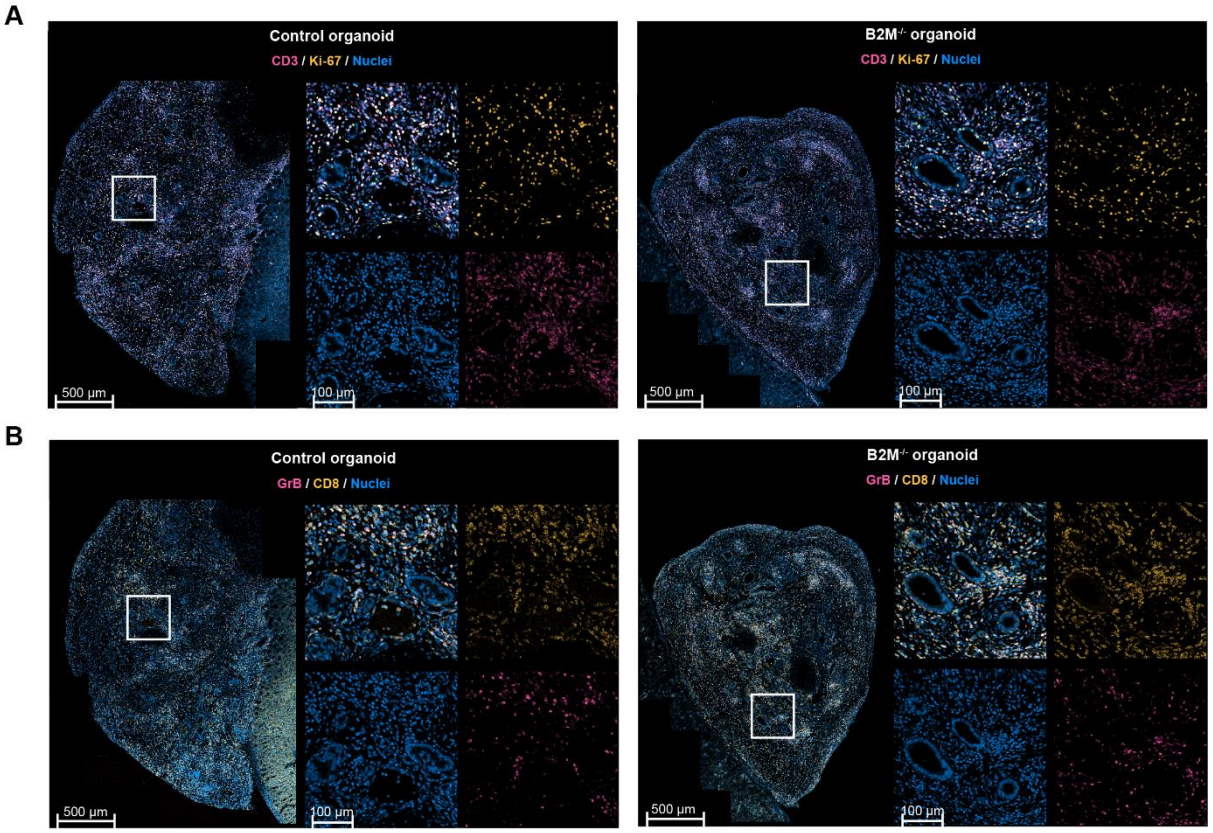

Figure S4. T cell proliferation and cytotoxic activity in transplanted kidney organoids

A and **B**) Representative fluorescent images of control and B2M$^{-/-}$ kidney organoids transplanted in the same mouse stained for **A**) CD3 (pink) and Ki-67 (yellow), and **B**) Granzyme B (pink) and CD8 (yellow). For each image a magnification is shown of the region indicated with a white square, including the separate fluorescent channels.

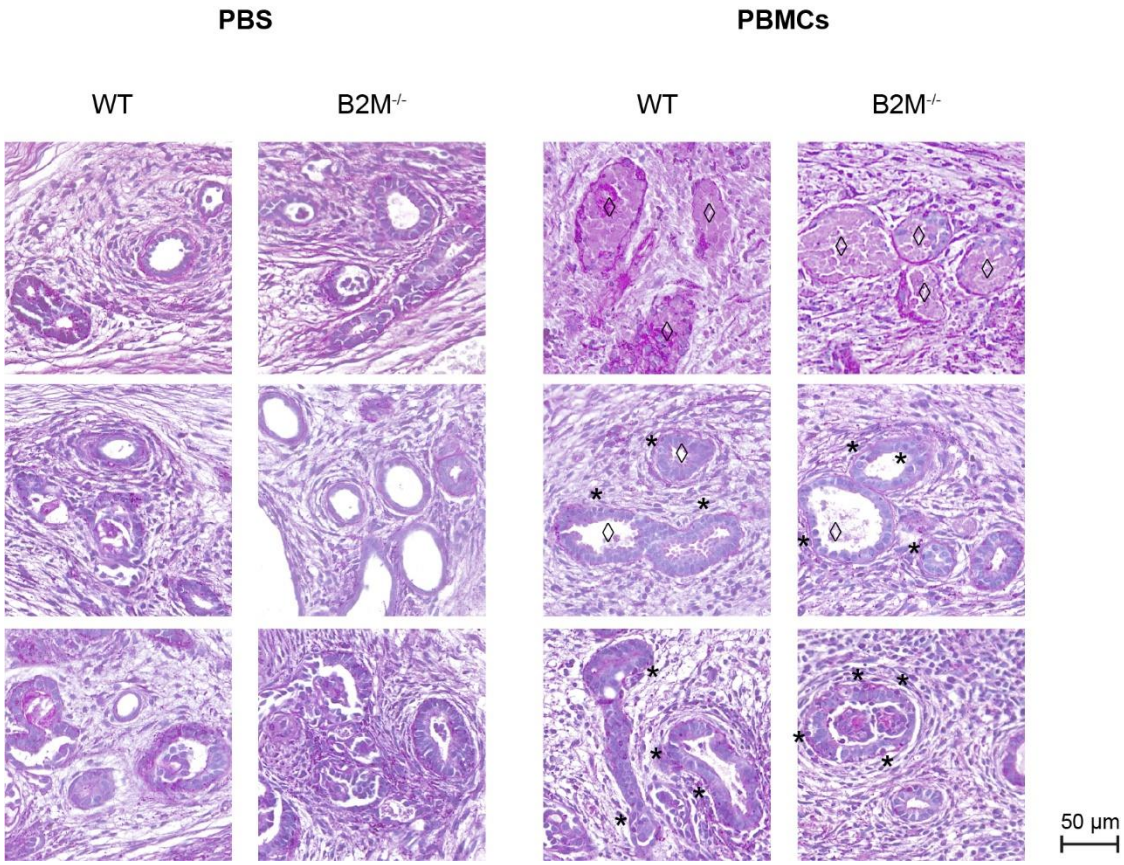

Figure S5. Tissue morphology in transplanted kidney organoids

Representative images of PAS stained transplanted kidney organoid sections of organoids collected at day 35 after transplantation. Pictures on the left show control and B2M$^{-/-}$ organoids of 3 separate mice injected with PBS and pictures on the right of 3 mice injected with PBMCs. Control and B2M$^{-/-}$ pictures next to each other originate from the same mouse. Locations where T cells have infiltrated the tubular wall (tubulitis) are indicated with *, and loss of tubular integrity (cytotoxicity) with ◊.

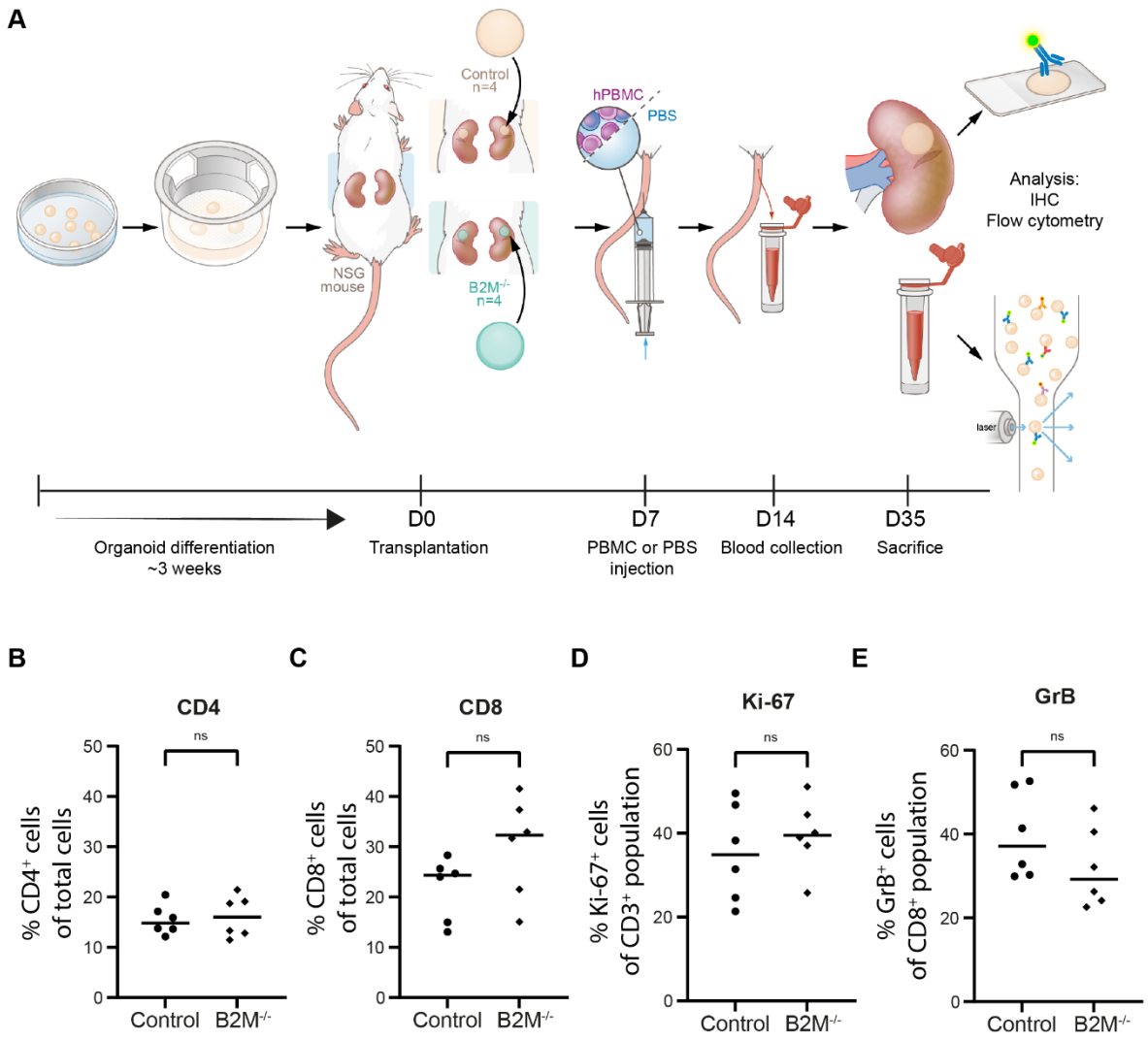

Figure S6. Rejection upon separate transplantation of control and B2M$^{-/-}$ kidney organoids

A) Schematic of the *in vivo* experimental model with transplantation of either control or B2M$^{-/-}$ organoids on both kidneys in each mouse. 4 mice were transplanted with control organoids and 4 other mice were transplanted with B2M$^{-/-}$ organoids. 1 week after transplantation, 2 mice were injected with PBS (1 control and 1 B2M$^{-/-}$ transplanted), and 6 mice were injected with PBMCs (3 control and 3 B2M$^{-/-}$ transplanted).

B-**E**) Quantification in stained organoid sections of **B**) the proportion CD4^{+} cells of the total cell population, **C**) the proportion CD8^{+} cells of the total cell population, **D**) the proportion Ki-67^{+} cells in the CD3^{+} cell population, and **E**) the proportion Granzyme B (GrB)$^{+}$ cells in the CD8^{+} cell population analysed by Qupath. For both control and B2M$^{-/-}$, individual results and mean are shown of 6 organoid sections (3 mice with each 2 transplants) and significance was evaluated using an unpaired T-test (ns = not significant).

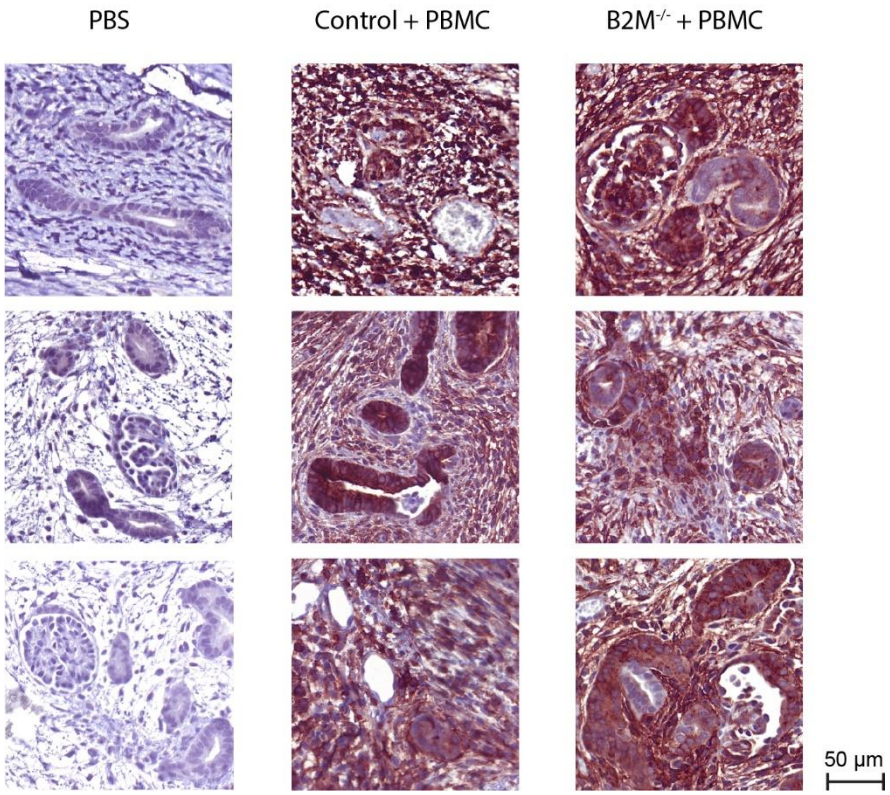

Figure S7. HLA class II expression in transplanted control and B2M$^{-/-}$ kidney organoids

Representative images of control or B2M$^{-/-}$ kidney organoid sections of the separate transplantation experiment stained for HLA-DR/DP/DQ (brown). Left images show 3 separate control organoids transplanted in mice injected with PBS. The middle images show 3 separate control organoids, and the right images 3 separate B2M$^{-/-}$ organoids transplanted in mice that were injected with PBMCs.

Table S1. Primers used for genomic PCR and RT-qPCR

Primer set	Primer	Sequence 5' - 3'
Genomic PCR primers		
1	B2M uArm FW	GGCAGATGCAGTCCAAACTCT
	GFP RV	CGTTGGGGTCTTTGCTCAGGG
2	puroR FW	GCTCGGCTTCACCGTCAC
	B2M dArm RV	GCTCTGGAGAATCTCACGCA
RT-qPCR primers		
GAPDH	FW	ACAGTCAGCCGCATCTTCTT
	RV	AATGAAGGGGTCATTGATGG
B2M	FW	TCGCGCTACTCTCTCTTTCTG
	RV	TTCTCTGCTGGATGACGTGAG
GFP	FW	ACCCCGACCACATGAAGCAGC
	RV	CGTTGGGGTCTTTGCTCAGGG
TAP1	FW	TCAGGGCTTTCGTACAGGAG
	RV	TCCGGAAACCGTGTGTACTT

Table S2. HLA haplotypes of control iPSCs and 3 PBMC donors

	iPSC	PBMC1	PBMC2	PBMC3
A	A*02:01	A*11:01	A*02:01	A*01:01
	A*32:01	A*24:02	A*68:01	A*26:01
B	B*15:18	B*15:01	B*13:02	B*08:01
	B*15:220	B*37:01	B*14:02	B*38:01
C	C*07:04/07:181	C*03:03	C*02:02	C*07:01
	C*12:03/12:28	C*06:02	C*06:02	C*12:03
DRB1	DRB1*03	DRB1*08	DRB1*07	DRB1*03
	DRB1*08	DRB1*13	DRB1*11	DRB1*13:
DRB3	DRB3	DRB3*02	DRB3*02	DRB3*01
			DRB4*01	
DQB1	DQB1*02	DQB1*04	DQB1*02	DQB1*02
	DQB1*04	DQB1*06	DQB1*03	DQB1*06

HLA typing was performed for HLA-A, -B, -C, -DRB1, -DRB3, and -DQB1. Matching HLA type of PBMC and iPSC donor are indicated in green. The degree of similarity in HLA type between PBMC donors and iPSC donor is indicated in the iPSC column (red is no match, orange is 1 matching donor, yellow is 2 matching donors).

Table S3. Antibodies used in flow cytometry and immunohistochemistry

Antibody	Dilution	Product code, company
Flow cytometry		
PE-conjugated mouse anti- HLA-ABC	1:100	555553, BD Biosciences
APC-conjugated anti-CD137	1:20	550890, BD Biosciences
APC-conjugated anti-CD54 (ICAM-1)	1:80	353112, Biolegend
PB-conjugated mouse-anti-human CD45	1:50	MCA87PB, Serotec
FITC-conjugated mouse-anti-human CD3	1:50	345763, BD Biosciences
PE-conjugated mouse-anti-human CD4	1:20	555347, BD Biosciences
PerCP-Cy5.5-conjugated anti-CD8	1:200	344710, Biolegend
V500-conjugated mouse-anti-human CD14	1:50	562693, BD Biosciences
AF700-conjugated mouse-anti-human CD19	1:50	56-0199, eBioscience
APC-conjugated mouse-anti-human CD56	1:50	17-0567-42, eBioscience
APC-Cy7-conjugated mouse-anti-mouse CD45.2	1:100	560694, BD Biosciences
Immunohistochemistry primary antibodies		
sheep anti-human NPHS1	1:100	AF4269, R&D Systems
mouse anti-E-Cadherin	1:250	610181, BD
biotinylated Lotus Tetragonolobus Lectin (LTL)	1:300	B-1325, Vector Laboratories
rabbit anti-CD3e	1:200	MA1-90582, Invitrogen
rabbit anti-CD4	Ready to use	5552737001, Roche
mouse anti-CD8	1:200	M7103, DAKO
mouse anti-Ki-67	1:200	M7240, DAKO
rabbit anti-Granzyme B (GrB)	1:100	262R-15-RUO, Sigma-Aldrich
mouse anti-HLA-DP, DQ, DR	1:100	M0775, DAKO
Immunohistochemistry secondary antibodies		
donkey anti-sheep Alexa-647	1:500	A21448, Invitrogen
donkey anti-mouse Alexa-488	1:500	A21202, Invitrogen
donkey anti-rabbit Alexa-568	1:500	A10042, Invitrogen
Streptavidin conjugated with Alexa-532	1:500	S11224, Invitrogen
HRP conjugated goat anti-mouse	1:200	P0447, DAKO

Reference

1. PathCards, pathway unification database; markers of kidney cell lineage. Weizmann institute of Science; Updated 2023 May 31; Version 5.16.984.0. Available from: https://pathcards.genecards.org/Card/markers_of_kidney_cell_lineage?queryString=kidney%20marker